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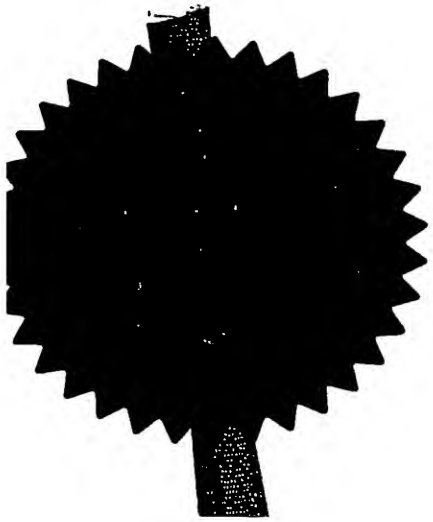

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2. **Patent application number** *(The Patent Office will fill in this part)* — 0323979.5

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P01/7700 0.00-0323979.5

3. **Full name, address and postcode of the or of each applicant** *(underline all surnames)*

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Patents ADP number *(if you know it)* — 08732273001

If the applicant is a corporate body, give country/state of incorporation — Sweden

4. **Title of the invention** — Therapeutic applications for C-peptide

5. **Name of your agent** *(if you have one)* — Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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No

81642.622

Therapeutic applications for C-peptide

The present invention relates to administration of C-peptide in a once daily dose, for use in the treatment of diabetes and diabetic complications.

Insulin-dependent diabetes mellitus (IDDM), generally synonymous with type 1 diabetes, is the classical, life-threatening form of diabetes, the treatment of which was revolutionized by the discovery of insulin in 1922. The prevalence of type 1 diabetes is unfortunately widespread throughout much of the world and hence type 1 diabetes represents a serious condition with a significant drain on health resources.

The etiology of type 1 diabetes is multifactorial and not yet entirely clear. However it is characterised by a partial or complete autoimmune destruction of the pancreatic beta cells. In the acute phase of type 1 diabetes insulin deficiency is thus the dominating pathophysiological feature.

After starting insulin treatment many patients enjoy good blood glucose control with only small doses of insulin. There is an early phase, the "honeymoon period", which may last a few months to a year and which probably reflects a partial recovery of beta cell function. This is, however, a temporary stage and ultimately, the progressive destruction of the beta cells leads to complete cessation of insulin secretion and increasing requirements for exogenous insulin.

While the short term effects of hypoinsulinemia in the acute phase of type 1 diabetes can be well controlled by insulin administration, the long term natural history of type 1 diabetes is darkened by the appearance in many patients of potentially serious complications known as late, or late onset complications. These include the specifically diabetic

problems of nephropathy, retinopathy and neuropathy. These conditions are often referred to as microvascular complications even though microvascular alterations are not the only cause. Atherosclerotic disease of the large arteries, particularly the coronary arteries and the arteries of the lower extremities, may also occur.

Nephropathy develops in approximately 35% of type 1 diabetes patients, particularly in male patients and in those with onset of the disease before the age of 15 years. Diabetic nephropathy is characterized by persistent albuminuria secondary to glomerular capillary damage, a progressive reduction of the glomerular filtration rate and eventually, end stage renal failure requiring dialysis treatment or kidney transplantation.

The prevalence of diabetic retinopathy is highest among young-onset type 1 diabetes patients and it increases with the duration of the disease. Proliferative retinopathy is generally present in about 25% of the patients after 15 years duration and in over 50% after 20 years. The earliest lesion of diabetic retinopathy is a thickening of the capillary basement membrane, followed by capillary dilation and leakage and formation of microaneurysms. Subsequently, occlusion of retinal vessels occurs resulting in hypoperfusion of parts of the retina, oedema, bleeding and formation of new vessels as well as progressive loss of vision.

The diabetes-induced nerve disorder is most often a distal symmetric primarily sensory neuropathy affecting 30-50% of type 1 patients. It is often associated with autonomic dysfunction. Sensory neuropathy may cause loss of sensation, appearance of paraaestesia or numbness or, alternatively, result in unpleasant sensations, sometimes pain, in the legs, feet or hands. The morphological changes of diabetic peripheral neuropathy include distal axonal loss with a reduction of the number of large (myelinated) and small fibers, focal demyelinisation and regenerating activity. The

function abnormalities include slowing of nerve conduction velocities, reduction of nerve signal amplitudes and rises in sensory modality thresholds. Autonomic neuropathy afflicts approximately 50% of the patients with type 1 diabetes of more than 15 years duration. It may evolve through defects in thermoregulation, impotence and bladder dysfunction followed by cardiovascular reflex abnormalities. Late manifestations may include generalized sweating disorders, postural hypotension, gastrointestinal problems and reduced awareness of hypoglycemia. The latter symptom has grave clinical implications.

A number of theories have been advanced with regard to possible mechanism(s) involved in the pathogenesis of the different diabetic complications but this has not yet been fully elucidated. Metabolic factors may be of importance and it has been shown that good metabolic control is accompanied by significantly reduced incidence of complications of all types. Nevertheless, after 7-10 years of good metabolic control, as many as 15-25% of the patients show signs of beginning nephropathy, 10-25% have symptoms of retinopathy and 15-20% show delayed nerve conduction velocity indicating neuropathy. With longer duration of the disease the incidence of complications increases further. There is thus a significant clinical need for the control and management of these diabetic complications.

Proinsulin C-peptide is a part of the proinsulin molecule which, in turn, is a precursor to insulin formed in the beta cells of the pancreas. For a long time it was believed that C-peptide (known variously as C-peptide or proinsulin C-peptide) had no role other than as a structural component of proinsulin, facilitating correct folding of the insulin part. However, it has in more recent years been recognised that C-peptide has a physiological role as a hormone in its own right (Wahren *et al.*, (2000), Am. J. Physiol.

Endocrinol. Metab, 278, E759-E768). In diabetic patients, it alleviates renal dysfunction, improves blood flow in several tissues, ameliorates nerve functional impairments and is believed to delay or prevent the onset of late complications (Wahren *et al.*, (2000) supra; Johansson J et al. Biochem Biophysical Research Comm. 2002; 295:1035-1040; Wahren et al. in International Textbook of Diabetes, 3rd Edition, editors DeFronzo, Ferrannini, Keen and Zimmet, 2003, Wiley, London). Indeed, C-peptide has been proposed for use in the treatment of diabetes in EP 132769 and in SE460334 for use in combination with insulin in the treatment of diabetes and prevention of diabetic complications.

C-peptide is known to have a relatively short half-life. In humans the half-life is approximately 30 minutes and a dose of C-peptide injected into a rat would be expected to have disappeared entirely from circulation within 2-3 hours. Due to the short half-life of C-peptide, in all prior art disclosures several daily doses (typically 3 or 4) or a continuously administered dose are used to treat diabetes or diabetic complications. For example, Sima et al. (Diabetologia, 44, 889-897, 2001) administered C-peptide in a continuous dose by osmopump to diabetic rats. In one known case Ido et al. (Science, 277, 563-566, 1997) administered C-peptide (at 130 nmol per kilogram of bodyweight) twice daily to rats with streptozotocin-induced diabetes. However, in this study human C-peptide was given to rats in a dose approximately five fold higher than otherwise used. Human C-peptide can be expected to be catabolized more slowly in the rat than the homologous C-peptide, which together with the high dosage may account for the observed effect in this study.

Similarly, insulin, which is derived from the same prohormone (proinsulin) as C-peptide requires administration 3-5 times daily.

The inventors of the present application have now surprisingly found that C-peptide given in a once daily dose can be used to treat type I diabetes and diabetic complications effectively. This is particularly surprising since a once daily administration of C-peptide would be expected to leave an animal without detectable C-peptide levels for at least 18-20 hours per day (approximately 75-80% of the time). Thus a new mechanism of action for C-peptide is suggested as it is not believed that the C-peptide remains in the plasma at levels which are sufficient to explain the beneficial effects. This finding offers significant practical advantages to doctor and patient alike in the treatment of diabetes and diabetic complications.

Thus, in one aspect the present invention provides a use of C-peptide or a functionally equivalent fragment, derivative or variant thereof in the manufacture of a medicament for administration to a patient as a once daily dose for the treatment of type I diabetes and/or diabetic complications. 'Diabetic complications' refer to the complications of type I diabetes. Many of the complications are linked to a reduction in Na^+K^+ATPase activity and therefore in a further embodiment, the invention provides the use of C-peptide or a functionally equivalent fragment, derivative or variant thereof in the manufacture of a medicament for administration to a patient as a once daily dose for use in stimulating Na^+K^+ATPase in a patient and/or for treating or preventing conditions associated with a sub-normal Na^+K^+ATPase activity.

Alternatively viewed, the invention provides the use of C-peptide or a functionally equivalent fragment, derivative or variant thereof in the manufacture of a medicament for the treatment of type I diabetes and/or diabetic complications, characterised in that the medicament is for once daily administration to a patient. Also, the invention provides C-peptide or a

functionally equivalent fragment, derivative or variant thereof for use as a once daily dose in therapy, in particular in the treatment of type I diabetes and/or diabetic complications.

The present invention also provides the use of C-peptide or a functionally equivalent fragment, derivative or variant thereof in the manufacture of a medicament for the treatment of type I diabetes and/or diabetic complications by administration once a day. Preferably the invention provides the use of C-peptide or a functionally equivalent fragment, derivative or variant thereof in the manufacture of a medicament in the form of an aqueous solution for the treatment of type I diabetes and/or diabetic complications by administration (of said medicament) once a day.

Reference to a 'once daily dose' or 'once daily administration' means, of course, not only that the medicament itself is only given once per day but that the patient receives no other C-peptide treatment. Such instructions may be made clear by the prescribing physician and/or in literature accompanying the packaged medication. The medicament may be adapted for once daily administration. This does not imply the presence of substances which act as release rate controlling agents, indeed the most preferred formulation of the invention is an uncompromised aqueous solution, but it may be reflected in the amount of active agent present, more than would typically be present in a dose prepared for thrice daily administration for example. Specific doses are described below.

"C-peptide" or "pro-insulin C-peptide" as used herein covers C-peptide isolated from any species. Preferably, "C-peptide" refers to human C-peptide having the amino acid sequence EAEDLQVGQVELGGGPGAGSLQPLALEGSLQ (SEQ ID NO. 1).

The term "fragment" as used herein includes fragments of a C-peptide provided that the fragment

retains the biological or therapeutically beneficial activity of the whole molecule. Preferred fragments comprise residues 15-31 of native C-peptide, more especially residues 20-31. Peptides comprising the pentapeptide EGSLQ (SEQ ID NO. 2) (residues 27-31 of native human C-peptide) are also preferred. The fragment may thus vary in size from e.g. 4 to 30 amino acids or 5 to 20 residues.

Suitable fragments are disclosed in WO 98/13384 the contents of which are incorporated herein by reference. Representative fragments include ELGGGPGAG (SEQ ID NO. 3), EGSLQ (SEQ ID NO. 2), ELGG (SEQ ID NO. 4), ELGGGP (SEQ ID NO. 5), GGPGA (SEQ ID NO. 6) or GSLQ (SEQ ID NO. 7). The fragment may also include an N-terminal fragment of C-peptide, typically having the sequence EAEDLQVGAVEL (SEQ ID NO. 8), or a fragment thereof which comprises 2 acidic amino acid residues, capable of adopting a conformation where said two acidic amino acid residues are spatially separated by a distance of 9-14Å between the α-carbons thereof. Also included are fragments having N and/or C-terminal extensions or flanking sequences. The length of such extended peptides may vary, but typically are not more than 50, 30, 25 or 20 amino acids in length.

Nevertheless, C-peptide itself, especially human C-peptide is particularly preferred. Also peptides very closely based on SEQ ID No. 1, e.g. those incorporating 1-4 additional or deleted amino acids or amino acid substitutions, such substitutions preferably being conservative.

The term "derivative" as used herein refers to C-peptide sequences or fragments thereof, which have modifications as compared to the native sequence.

Such modifications may be one or more amino acid deletions, additions, insertions and/or substitutions. Chemical modification of the peptide structure is not precluded e.g. by glycosylation as long as the structure

of the derivative remains essentially peptide in nature. As mentioned above, modification of an amino acid sequence may be by amino acid substitution, for example an amino acid may be replaced by another which preserves the physicochemical character of the peptide (e.g. A may be replaced by G or vice versa, V by A, L or G; E by D or vice versa; and Q by N). Generally, the substituting amino acid has similar properties e.g. hydrophobicity, hydrophilicity, electronegativity, bulky side chains etc. to the amino acid being replaced. Isomers of the 'native' L-amino acid, e.g. D-amino acids may be incorporated.

Additional variants may include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acids. Longer peptides may comprise multiple copies of one or more of the peptide sequences. C and N-terminal protecting groups may be included.

Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced at a site in the protein. Deletional variants are characterised by the removal of one or more amino acids from the sequence.

"Variants" may include, for example, different allelic variants as they appear in nature, e.g. in other species or due to geographical variation etc.

The variants, derivatives and fragments are functionally equivalent in that they exhibit at least 40%, preferably at least 60%, more preferably at least 80% of the activity of proinsulin C-peptide, particularly human C-peptide. Thus are capable of functioning as proinsulin C-peptide i.e. can substitute for C-peptide itself.

Such activity means any activity exhibited by a native C-peptide, whether a physiological response exhibited in an *in vivo* or *in vitro* test system, or any biological activity or reaction mediated by a native C-

peptide, for example in an enzyme assay or in binding to test tissues or membranes.

Thus, it is known that C-peptide increases the intracellular concentration of calcium. An assay for C-peptide activity can thus be by assaying for changes in intracellular calcium concentrations upon addition or administration of the peptide (e.g. fragment or derivative) in question. Such an assay is described in for example in Ohtomo *et al.*, (1996), supra, Kunt *et al.*, supra; Shafqat *et al.*, supra and in Example 1 below.

Further, C-peptide has been found to induce phosphorylation of the MAP-kinases ERK 1 and 2 of a mouse embryonic fibroblast cell line (Swiss 3T3), and measurement of such phosphorylation and MAPK activation may be used to assess, or assay for C-peptide activity, as described for example by Kitamura *et al.*, supra and in Example 2.

C-peptide also has a well known effect in stimulating Na^+K^+ATPase activity and this also may form the basis of an assay for C-peptide activity, for example as described in WO 98/13384 or in Ohtomo *et al.*, (1996), supra or Ohtomo *et al.*, (1998), supra. This is the preferred test to establish 'C-peptide-like' activity and active peptides will preferably induce NA^+K^+ATPase activity in the sciatic nerve by at least 50% over basal levels.

An assay for C-peptide activity based on endothelial nitric oxide synthase (eNOS) activity is also described in Kunt *et al.*, supra, using bovine aortic cells and a reporter cell assay.

Binding to particular cells may also be used to assess or assay for C-peptide activity, for example to cell membranes from human renal tubular cells, skin fibroblasts and saphenous vein endothelial cells using fluorescence correlation spectroscopy, as described for example in Rigler *et al.*, supra, Henriksson *et al.*,

supra and Pramanik *et al.*, supra. Finally, affinity tests based on measurements of protein binding may be used as activity tests of C-peptide.

The "patient" can be any animal but preferably is a human.

Administration of the single daily dose may be by any suitable method known in the medicinal arts, including oral, parenteral, topical, subcutaneous administration or by inhalation. Preferably administration is by subcutaneous administration. The single dose may be administered at any time during the day. For humans, typically, the dosage used is 3-30 nmol/kg of bodyweight/24 hours of C-peptide. Preferably the dose used is 5-20 nmol/kg of bodyweight/24 hours. Thus a single dose as administered will vary depending on the weight of the patient but may typically be in the region of 1200 to 1800 nmol, e.g. about 1500 nmol.

The dose may or may not be in solution. If the dose is administered in solution, it will be appreciated that the volume of the dose may vary, but will typically be 10 μl - 1 ml. Preferably the dose will be given in a volume of 900 μl, 800 μl, 700 μl, 600 μl, 500 μl, 400 μl, 300 μl, 200 μl, 100 μl, 50 μl or 20 μl.

The C-peptide dose in solution can also comprise a preservative and/or a buffer. For example, the preservative m-cresol can be used. Typical concentrations of preservatives include 0.5 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml or 5 mg/ml. Examples of buffers that can be used include sodium phosphate buffer (pH 7.3) or sodium bicarbonate buffer (pH 7.3). It will be appreciated that the C-peptide dose may comprise of one or more of C-peptide, fragments, derivatives or functionally equivalent variants of C-peptide. Hence, the single dose may comprise human C-peptide and the C-peptide fragment EGSLQ and/or the N-terminal C-peptide fragment.

In a further embodiment, the present invention

provides the use of a pharmaceutical composition comprising C-peptide or a functional equivalent fragment, derivative or variant thereof in the manufacture of a medicament for administration to a patient as a once daily dose for the treatment of type I diabetes and/or diabetic complications.

Pharmaceutical compositions for use in the present invention may be formulated according to techniques and procedures well known in the art and widely discussed in the literature and may comprise any of the known carriers, diluents or excipients. The compositions may be in the form of (sterile) aqueous solutions and/or suspensions of the pharmaceutically active ingredients, aerosols, ointments and the like. Formulations which are aqueous solutions are most preferred. Such formulations typically contain the peptide itself, water and one or more buffers which act as stabilisers (e.g. phosphate containing buffers) and optionally one or more preservatives. Such formulations containing, e.g. 1200 to 1800 nmol of the peptide constitute a further aspect of the invention.

Compositions to be used in the invention suitable for parenteral administration may comprise sterile aqueous solutions and/or suspensions of the pharmaceutically active ingredients preferably made isotonic with the blood of the recipient, generally using sodium chloride, glycerin, glucose, mannitol, sorbitol and the like.

Compositions of the invention suitable for oral administration may for example comprise peptides in sterile purified stock powder form preferably covered by an envelope or envelopes (enterocapsules) protecting from degradation of the peptides in the stomach and thereby enabling absorption of these substances from the gingiva or in the small intestines.

The total amount of active ingredient in the composition may vary from 99.99 to 0.01 percent of

weight.

As discussed above, the C-peptide dose and pharmaceutical compositions containing this dose can be used in the manufacture of a medicament to treat Type I diabetes and diabetic complications. Such diabetic complications include retinopathy, nephropathy and neuropathy. The administration of the single C-peptide dose can hence be used to improve nerve cell structure (paranodal swelling, paranodal demyelinsation, intercalated internodes, Wallerian degeneration, regeneration, sequential demyelinisation and excessive wrinkling). An improvement in Na^+K^+ATPase activity may also be observed. 'Treatment' includes a measurable improvement in one or more of these complications or in the parameters which define type I diabetes e.g. reduction in urinary albumin excretion, reduction or maintenance of plasma creatinine concentration, maintenance or increase in glomerator filtration rate, or improvement in retinal-vitreous body leakage, aneurysm formation or retinal breeding. The once-daily therapeutic regimen described herein may, optionally in combination with conventional insulin therapy, be useful in preventing or substantially retarding the development of the late diabetic complications discussed above. Thus there may be a prophylactic element to the 'treatments' discussed above.

In a further aspect the invention provides a therapeutic regimen for the treatment of type I diabetes and/or the complications thereof wherein C-peptide or a functionally equivalent fragment, derivative or variant thereof is administered once daily to a patient.

In another embodiment, the invention provides a method of treating type I diabetes and/or diabetic complications in a patient comprising administering C-peptide or a functionally equivalent variant fragment or derivative thereof in a once daily dose. Methods of treatment corresponding to the various additional uses

discussed above are further aspects of the invention.

The method also covers a method of treatment comprising administering one dose of a pharmaceutical composition of the invention.

The invention will now be described in more detail in the following non-limiting Examples and with reference to the drawings in which:

Figure 1 is a graph showing changes in motor nerve conduction velocity (MNCV) expressed in m/s in healthy control and diabetic animals receiving C-peptide treatment for 8 weeks with different dosing regimens; diab = diabetic, s.c. = subcutaneous;

Figure 2 is a graph showing changes in sensory nerve conduction velocity (SNCV) expressed in m/s in healthy control and diabetic animals receiving C-peptide treatment for 8 weeks with different dosing regimens;

Figure 3 is a graph showing Na^+K^+ATPase activity in the sciatic nerve (μmol ADP/mg/h) in healthy control and diabetic rats receiving C-peptide treatment for 8 weeks with different dosing regimens; and

Figure 4 is a table showing teased fiber assessment of nodal and axonal morphometric changes in healthy controls and diabetic rats receiving C-peptide treatment for 8 weeks with different administration regimens.

EXAMPLES

Example 1

Further technical details can be found in Sima et al., Diabetologia (2001) 44: 889-897.

Prediabetic male BB/Wor rats were used. Ten rats were used in each group, where the animals were closely age-matched. They were maintained in metabolic cages with free access to water and rat chow. Bodyweight, urine volume and glucosuria were monitored daily to ascertain onset of diabetes. Immediately after the onset of diabetes, administration of C-peptides was commenced in either a single dose/24 hours, three doses/24 hours or as a continuous infusion by osmopump.

The single dose given was 75 nmol/kg and the three doses given were each 25 nmol/kg. The continuous infusion gave a dose of 75 nmol/kg over a 24 hour period.

Synthetic rat C-peptide II with a purity of more than 98% by HPLC was used (Genosys, Cambridge, UK) dissolved in saline (12 mg/ml). Control rats were given a dose of saline instead of C-peptide.

The nerve conduction velocity in motor and sensory nerves and Na^+K^+ATPase levels in the rats were measured. Baseline nerve conduction velocity was measured within 24 hours of onset of diabetes. Measurements were taken in the siatic-tibial nerves under temperature-controlled conditions (35°C-37°C) as in Sima et al. J. Clin. Invest. (1996) 97: 1900-1907.

To measure Na^+K^+ATPase activity and investigate nerve cell morphology, animals were anaesthetized with Na-pentobarbital (50 mg/kg bodyweight i.p.) and both

sciatic nerves were dissected, weighed and snap frozen in liquid nitrogen for measurements of nerve glucose, sorbitol, fructose and Na^+K^+ATPase activity. The right sural nerve was fixed in 7.5% glutar aldehyde in 0.1 mol/l cacodylate buffer at pH 7.40 and post fixed in 1% osmium tetroxide (pH 7.40). The distal sural nerve was used for teased fibre preparations.

Insulin and C-peptide concentrations. Serum insulin and C-peptide concentrations were examined using commercially available RIA kits (Linco Research, St. Charles, Mo., USA).

Biochemical analyses. For nerve glucose, sorbitol and fructose, sciatic nerve samples were homogenized in 2 ml of 5% TCA. Aldonitrile derivatives were formed by adding 0.3 ml hydroxylamine in pyridine-methanol 4:1 (vol:vol). Samples were sonicated for 1 mm and 1 ml of acetic anhydride and 2 ml of 1,2 dichlorothane were added and samples were washed in 1.0 N HCl. Samples were reconstituted in 2-butanane and analysed by gas-liquid chromatography.

For Na^+/K^+-ATPase activity, nerve samples were homogenized in 2 ml of 0.2 mol/l sucrose and 0.02 mol/l TRIS-HCl at pH 7.5. Between 10 and 20μl of the homogenate was assayed enzymatically for total ATPase in 1 ml of 100 mmol/l NaCl, 10 mmol/l KCl, 2.5 mmol/l $MgCl_2$, I mmol/l TRIS ATP, 1 mmol/l phosphoenolpyruvate, 30 mmol/l imidazole HQ buffer (pH 7.30), 0.15 mmol/l NADH, 50 pg lactate dehydrogenase and 30μg pyruvate kinase. To measure ouabain-inhibited ATPase, 20μl of 25 mmol/l of ouabain was added. Na^+/K^+-ATPase activity was defined as the difference before and after ouabain and was expressed as μmol ADP formed per gram of wet weight per hour.

Morphometric analysis. Semithin (0.5 μm) cross-sections of sural nerves were used for morphometric analysis. The following measurements of myelinated fibres were obtained: total number, axonal and myelin size (μm^2), fibre density (n/mm^2), coefficient of variance (CV) of fibre densities between image frames, fibre occupancy (% of endoneurial area), and axon to myclin ratio.

Teased fibre examinations. A mean of 168±4 myelinated fibres were teased from each sural nerve and scored for specific changes. The temporal sequence and increasing severity are represented by normality, paranodal swelling, paranodal demyelination, excessive myelin wrinkling, intercalated inter-nodes, segmental demyelination, Wallerian degeneration, and regeneration. Changes were expressed as percentages of total fibres.

Statistical analysis. The results are presented as means ± SE and the significance of differences was calculated by analysis of variance (ANOVA). Group differences were assessed by post hoc analysis using the Student-Newman-Keuls test. Tissue samples for biochemical, morphometric and teased fiber analyses were coded to mask animal identity. A p value of less than 0.05 was considered statistically significant.

Results

The results can be seen in Figures 1 to 4. The results show that nerve conduction velocity and Na^+K^+ATPase activity is clearly and sufficiently improved in rats given a once daily dose of C-peptide and that the once daily dose is as effective as three daily dose or a continuous infusion.

The morphometric data also show improved morphology of cells in rats given a once daily dose of C-peptide compared to diabetic rats.

Claims

1. Use of C-peptide or a functionally equivalent fragment, derivative or variant thereof in the manufacture of a medicament for administration to a patient as a once daily dose for the treatment of type I diabetes and/or diabetic complications.

2. Use according to claim 1 wherein the C-peptide is human C-peptide.

3. Use according to any one of claims 1 to 3 wherein the patient is a human.

4. Use according to any one of claims 1 to 4 wherein the medicament contains 1200 to 1800 nmol of C-peptide or a fragment, derivative or variant thereof.